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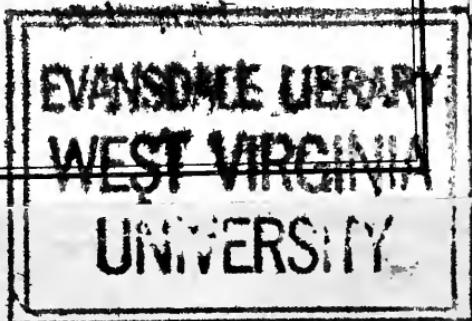
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WEST VIRGINIA UNIVERSITY
AGRICULTURAL EXPERIMENT STATION
MORGANTOWN, W. VA.

BULLETIN 134

JUNE, 1911

Experiments in the Production of Sanitary Milk.

HORACE ATWOOD AND N. J. GIDDINGS.

[The Bulletins and Reports of this Station will be mailed free to any citizen of West Virginia upon written application. Address Director of Agricultural Experiment Station, Morgantown, W. Va.]

neck. They were then sterilized in an oven at 190° to 200° C for 2 to 5 minutes, and were used within a few days.

In some cases the entire milk from a given quarter was taken as a sample and enamel ware milk cans of 1 gallon capacity were used for such samples. The cover of each can was wrapped in cotton and placed in a small sheet iron box, while the top of the can was carefully covered with two sheets of paper tied on. The cans and covers were sterilized in an oven as described above.

Samples were received at the laboratory within $1\frac{1}{2}$ to $2\frac{1}{2}$ hours after taking. They were thoroughly shaken, using both a rotary and a vertical movement. Dilutions were made into flasks of sterile distilled water and shaken, using rotary movements. One cubic centimeter of this dilution was added to 10 c. c. of agar or gelatin, and plates poured at once. In all cases, unless otherwise stated, there was one agar plate and one gelatin plate poured for each dilution. The plates were incubated at 18° to 20° C and counts made after 3 to 4 days. In some cases the number of colonies on a plate was too great to count, and a portion, 1 square centimeter to one-fifth of the plate area was counted, and the total number of colonies estimated from that. It will be seen that the bacterial content stated for nearly every sample is an average of counts from four poured plates.

THE MILK COOLER AND BOTTLING MACHINE AS A SOURCE OF BACTERIAL CONTAMINATION

The cooling apparatus employed is of the usual type and is shown in Fig. 1. During the tests reported in tables I and II it was washed as is customary, first with lukewarm water, then with hot water to which washing compound was added, and finally rinsed with scalding water.

In taking the samples of uncooled milk a 20 quart can of warm milk was poured into the vat located above the milk cooler and the sample taken at once by means of a sterile cup. The milk was then allowed to run over the cooler and the samples of cooled milk taken after the milk had collected in the tank of the bottling machine. The samples of uncooled milk were then placed in ice water for a time so as to cool the milk to practically the same temperature as the other samples.

The following table shows the germ content of the milk before and after it was run over the cooler. In these tests the cooler was freely exposed to the air of the dairy room.

Table I Bacterial Counts of Milk Before and After Passing Over Cooler Nov. 20-26, 1907.*

BACTERIA

		MOULDS							
		BACTERIA			MOULDS			Remarks	
Sample	Bacteria Total	Rapid Liquifiers	% Rapid Liquifiers	Slow Liquifiers	% Slow Liquifiers	Moulds	Moulds	Count is Probably	
11-20 Uncooled	1,240	65	5.5	33	2.2	325	Fairly accurate	3500 Low	Liquifiers forced early counting
11-20 Cooled	18,200	165	0.9	125	0.7	425	400 High		
11-21 Uncooled	1,580	65	4.2	185	11.5	100			
11-21 Cooled	4,300	190	4.5	100	2.3	450	200 High		
11-22 Uncooled	10,380	33	0.3	570	5.5	34	Fairly accurate	Too low	Early liquification of 2 plates
11-22 Cooled	39,630	290	0.7	285	0.7	125	Slightly low		
11-23 Uncooled	11,670	90	0.8	185	1.6	0	Low		
11-23 Cooled	13,800	65	0.5	50	0.4	135	Early liquification of 3 plates		
11-25 Uncooled	20,400	0	0.0	250	1.2	17	Accurate		
11-25 Cooled	17,400	0	0.0	800	4.7	25	Fairly accurate		
11-26 Uncooled	18,900	33	0.2	160	0.8	17	Trifle low		
11-26 Cooled Average	19,200	140	0.7	235	1.2	110	Trifle low		
Average Cooled	18,750								
Average Uncooled	10,700								

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*These determinations were made by Mr. C. P. Hartley while he was associated with this Station.

MILK BEFORE AND AFTER PASSING OVER COOLER

Table II Cooler Not Enclosed

DATE	No. of Sample	Treatment	Dilutions Used	Bacteria per cc.
April 24, 1909.....	1	Uncooled	250 & 500	229000
April 24, 1909.....	2	Cooled	250 & 500	254000
April 28, 1909.....	3	Uncooled	500 & 1000	4250
April 28, 1909.....	4	Cooled	500 & 1000	6100
April 29, 1909.....	5	Uncooled	500 & 825	7200
April 29, 1909.....	6	Cooled	500 & 1000	8200
May 3, 1909.....	7	Uncooled	500 & 1000	6400
May 3, 1909.....	8	Cooled	500 & 1000	9200
May 10, 1909.....	9	Uncooled	500 & 1000	1250
May 10, 1909.....	10	Cooled	500 & 1000	2100
May 17, 1909.....	11	Uncooled	250 & 500	1600
May 17, 1909.....	12	Cooled	250 & 500	1900
May 25, 1909.....	13	Uncooled	125 & 250	8000
May 25, 1909.....	14	Cooled	125 & 250	16000
May 26, 1909.....	15	Uncooled	125 & 250	4000
May 26, 1909.....	16	Cooled	125 & 250	4000

Average No. of bacteria in uncooled samples, 32,700.

Average No. of bacteria in cooled samples, 37,700.

These results show that in practically every case the number of bacteria in the milk was greater after it had been passed over the cooling apparatus than it was before. Since the prime object in using a milk cooler is to keep down the bacterial content, it would seem essential to prevent these germs from accumulating upon the apparatus itself. For the purpose of eliminating such bacteria as were not removed or destroyed by the ordinary washing processes, it seemed as though some method of applying live steam directly to the apparatus, would be most efficient and practical. A practically steam-tight compartment was therefore constructed so as to enclose the milk cooler and bottling machine. It was constructed in the form of a box, and was provided with suitable doors for the convenient operation and washing of the equipment. To prevent rusting the interior was lined with tinned sheet copper.

After the cooler and bottling machine had been enclosed, as shown by Figs. 2 and 3, instead of rinsing with scalding water live steam was used, the steam being turned into the little room or enclosure surrounding the cooler and bottling machine for about fifteen minutes. The apparatus was

steamed after it had been used and washed, and the doors of the enclosure were not opened until the cooler and bottle filler were to be used again.

Table III gives the results of the tests after the cooler and bottling machine had been enclosed.

MILK BEFORE AND AFTER PASSING OVER COOLER

Table III Cooler Enclosed

DATE	No. of Sample	Treatment	Dilutions Used	Bacetria per cc.
October 1, 1909...	17	Cooled	200 & 1000	17800
October 1, 1909....	18	Uncooled	200 & 1000	16800
	19-22	Omitted	as plates	spoiled.
December 12, 1909..	23	Cooled	125 & 500	930
December 12, 1909.	24	Uncooled	125 & 500	1800
December 28, 1909..	25	Cooled	50 & 200	600
December 28, 1909..	26	Uncooled	50 & 200	550
January 6, 1910....	27	Cooled	10 & 50	1450
January 6, 1910....	28	Uncooled	10 & 50	1400
January 15, 1910....	29	Cooled	10 & 50	3700
January 15, 1910....	30	Uncooled	10 & 50	4000

Average No. of bacteria in cooled sample, 4900.

Average No. of bacteria in uncooled sample, 4910.

It is evident from the above figures that the cooling and bottling apparatus ceased to be a source of contamination after it had been properly enclosed and given a careful steaming each day. In order to further test the efficiency of this treatment, a series of trials were planned allowing sterile water instead of milk to flow over the cooler. The method of procedure was as follows: A 2-liter glass stoppered bottle was carefully filled with boiling hot water, a bit of string placed inside the neck of the bottle and allowed to hang down 5 or 6 inches on the outside, and the stopper dropped in loosely against the string. The top of the bottle was carefully

wrapped with cotton, and then the bottle was placed in an autoclave and heated for 45 to 60 minutes at 15 to 20 pounds steam pressure. After the bottle was taken out and allowed to cool, the string between stopper and bottle was pulled out allowing the stopper to drop in tight so as to prevent slopping in carrying bottle. In removing the string, care was exercised not to touch either the stopper or disturb the cotton wrapping.

Four such bottles were used for each test. They were taken to the dairy within 24 to 48 hours after sterilization, unwrapped, and the contents of the four bottles poured into the little tank located immediately above the cooler. The water was then allowed to flow over the cooler into the bottling machine. After all of the water had collected in the tank of the bottling machine the mouth of a sterile sample bottle was pressed against one of the valves of the bottling machine and allowed to fill in the usual way.

The following table gives the bacterial content of the water after passing over the cooler and through the bottling machine.

Table IV Bacteria on Cooler

COOLER ENCLOSED AND STEAMED DAILY		COOLER LEFT OPEN AND MERELY WASHED AND SCALDED	
DATE	Bacteria per cc.	DATE	Bacteria per cc.
October 1, 1909....	2	April 22, 1910.....	1030
Ocotber 14, 1909....	1	April 23, 1910.....	3720
December 10, 1909..	1	April 27, 1910.....	3720
December 28, 1909..	1	April 28, 1910.....	16400
January 6, 1910....	8	April 29, 1910.....	21900
January 15, 1910....	1		
Average No.....	2	Average No.....	11400

From the three series of experiments previously described, we feel reasonably safe in concluding that the ordinary milk cooling devices are not nearly so efficient as they should be for keeping down the bacterial content of milk, but that by

properly enclosing such apparatus and subjecting it to live steam as described above, it ceases to be a source of contamination, and the beneficial effects of cooling are much greater.

It is evident from the results secured on Jan. 6, 1910, that the steam treatment was not continued quite long enough. It must also be remembered that the steaming does not in any way reduce the necessity for thorough washing. In order to secure good results the apparatus must be carefully enclosed so that the steam is retained in the chamber and the live steam must be permitted to run into the chamber for a sufficient length of time to heat the articles to the boiling point of water and maintain that temperature for 15 to 20 minutes.

In some cases it may be difficult to determine just how long steam ought to be admitted into the enclosure in order to sterilize the apparatus, but when in doubt it is a good practice to steam a few minutes longer. A potato the size of a hen's egg may be used as a fairly good indicator. If the potato is placed in an open dish midway between the ceiling and the floor of the enclosure, the steam should be admitted until it is fairly well cooked through. The length of time required for any sized enclosure and steam pressure can be determined in this way by one or two trials.

(A) RELATIVE NUMBER OF BACTERIA IN DIFFERENT PORTIONS OF MILK FROM SAME PART OF UDDER

A few tests were made in order to secure some data as to the relative numbers of bacteria occurring in the first milk drawn, in that taken when about half the milk had been secured, and in the stripplings. For this work, the sterile glass stoppered bottles were used and two sets of samples were taken for each test, one from the hind quarters and the other from the front quarters.

In taking the samples of milk directly from the udder great care was exercised to prevent bacteria from external sources entering the milk. The hair on the cow's udder and flanks was kept closely clipped, and immediately before each sample was taken the teats, udder and flank were thoroughly washed with an antiseptic solution, usually two per cent Chloronaphtholeum, and wiped with a sterile towel until no superfluous moisture remained. Then a paper cap was removed from a sample bottle and, while holding the bottle in a horizontal position with the right hand, the glass stopper was grasped by the index and middle finger of the left hand,

removed from the bottle, and carried underneath it, the left hand grasping the bottle, thus leaving the right hand free to do the milking. The sample bottles were maintained in as nearly a horizontal position as possible until the sample had been obtained and the stopper inserted. The paper cap was then put in place.

The first set of samples consisted of only one sample from the hind quarters and one sample from the front quarters, being portions of the entire milk secured from these quarters. The results are given in the following table.

Table V Bacteria in Different Portions of Milk From Same Quarters.

BACTERIA PER CUBIC CENTIMETER

Dilution	Hind Quarters			Front Quarters			Remarks
	50	1st Milk	2	50	1st Milk	2	
Date							
Feb. 26, '10	250	2080	Dilutions used here were 5 & 25
Mar. 2, '10	200	85	150	6000	1300	Front stripping omitted by mistake
Mar. 3, '10	200	14	50	2550	65	2200	
Mar. 5, '10	Less Than 50	38	100	2000	160	1150	
Mar. 7, '10	Less Than 50	3	75	4200	26	1600	

These tests serve to bring out the fact that the first milk and the strippings contain far more bacteria than that taken between these portions. It must not be assumed, however, that, since the strippings contain a larger number of germs, this portion of the milk should be left. Stocking has shown* that careful stripping keeps the bacterial content of milk low, while leaving a little milk in the udder from one milking to the next nearly doubled the number of bacteria per c. c. It is a matter of general belief among dairymen that the careless stripping of cows has a tendency to decrease the production of milk to a considerably greater extent than can be accounted for by the small amount of milk left in the udder at each milking. Whether this decreased yield is brought about through the increase in the bacterial content of the udder, or in some other way remains to be determined.

(B) RELATIVE NUMBERS OF BACTERIA IN DIFFERENT QUARTERS OF UDDER, AND RELATION OF BACTERIAL CONTENT AND MILK YIELD

Several series of samples were taken for the purpose of making some determinations as to the number and distribution of bacteria normally present in the cow's udder. The first set of samples were taken in gallon, enamel ware milk cans. These cans were sterilized and handled as described on page 84 and one can was used for each quarter. The samples for all other trials were taken in the glass stoppered bottles prepared as described on page 84.

For these smaller samples, an aliquot portion of the milk from each quarter was obtained by milking every third fourth, or fifth stream into the bottle, the other streams being milked into another receptacle.

On each of three dates the amount of milk from each quarter was carefully weighed, in order to learn if there was any relation between the amount of milk secreted and the number of bacteria per cubic centimeter.

The results of these tests are given in the following table:

*Stocking, W. A., Jr., Conn. (Storrs) Exp. Sta. Report 18 (1906) p. 85.

Table VI Number of Bacteria and Amount of Milk from Different Quarters.

Date	HIND QUARTERS				FRONT QUARTERS				Remarks	
	LEFT		RIGHT		LEFT		RIGHT			
	Dilutions Used	Bacteria per cc.	Dilutions Used	Bacteria per cc.	Dilutions Used	Bacteria per cc.	Dilutions Used	Bacteria per cc.		
Mar. 14, '10	5 & 25	60	5 & 25	55	20 & 100	625	20 & 100	8000	Entire milk taken for each sample.	
Mar. 17, '10	5 & 25	44	5 & 25	55	20 & 100	40	20 & 100	10500	Portion of milk taken for each sample.	
Mar. 18, '10	5 & 25	70	5 & 25	50	20 & 100	85	20 & 100	12100	Portion of milk taken for each sample.	
Mar. 21, '10	5 & 25	100	5 & 25	55	20 & 100	110	20 & 100	8400	Portion of milk taken for each sample.	
Mar. 22, '10	5 & 25	33	5 & 25	50	20 & 100	20	50 & 200	1900	Portion of milk taken for each sample.	
Mar. 26, '10	5 & 25	10 & 50	90	50 & 250	3500	Portion of milk taken for each sample.	
Mar. 29, '10	5 & 25	25	5 & 25	32	5 & 25	50	50 & 125	9900	Portion of milk taken for each sample.	
					Weight of Milk		Weight of Milk		Weight of Milk	
Mar. 14, '10	5.3 lb. or 2405 grams	5 lb or 2270 grams	5.6 lb. or 2542 grams	4.9 lb. or 2222 grams	2.52 lb. or 1140 grams		1.43 lb. or 650 grams		1.4 lb. or 636 grams	
Mar. 24, '10	5.6 lb. or 2542 grams	4.9 lb. or 2222 grams	5.85 lb. or 2656 grams	4.87 lb. or 2211 grams	1.95 lb. or 885 grams		1.52 lb. or 872 grams		1.52 lb. or 690 grams	

These figures show a very pronounced difference both in amount of milk, and bacteria per c. c. from the different quarters of the udder, but the order of differences exhibited in one table are reversed in the other. That is, where the number of bacteria is high the amount of milk is small and where the number of bacteria is small the amount of milk is large. In order to bring out this fact more clearly, and shown the approximate total number of bacteria contained in all the milk from any quarter, another tabulation is given below. The averages were obtained from the data given in the previous table.

As the average specific gravity of milk is about 1.035, the amount in grams was divided by that factor to determine the equivalent amount in cubic centimeters.

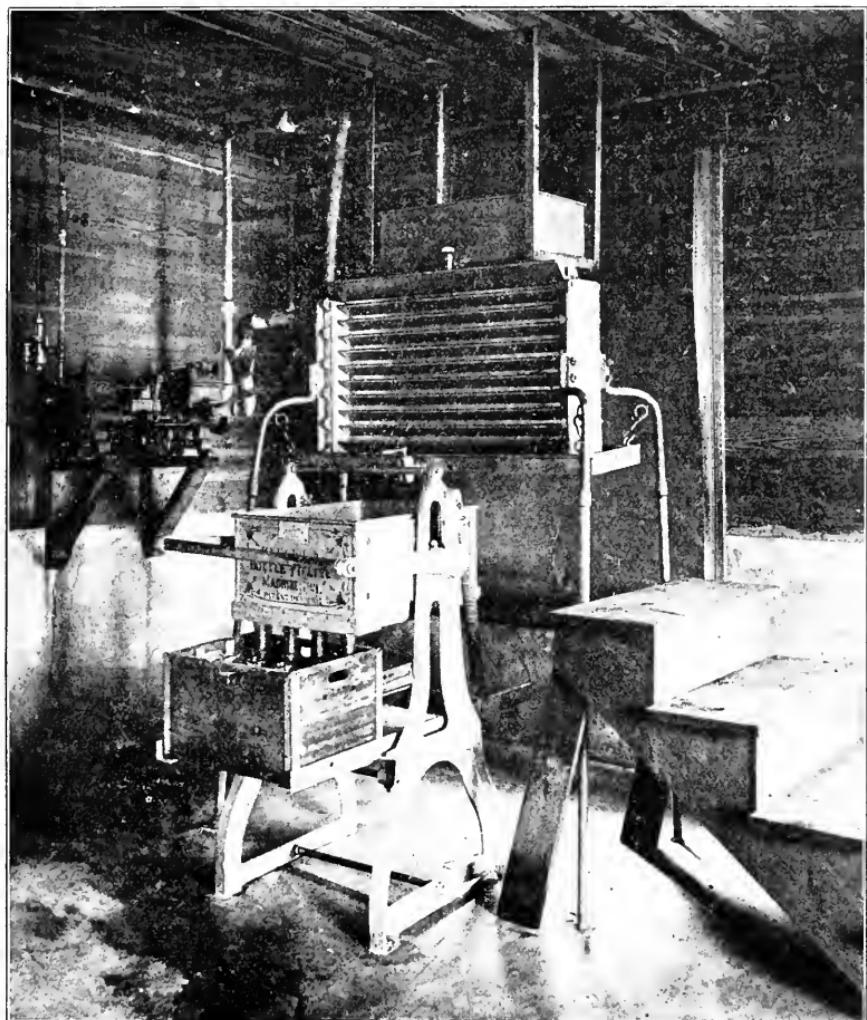


Fig. 1.—Milk Cooler and Bottling Machine as Originally Installed.

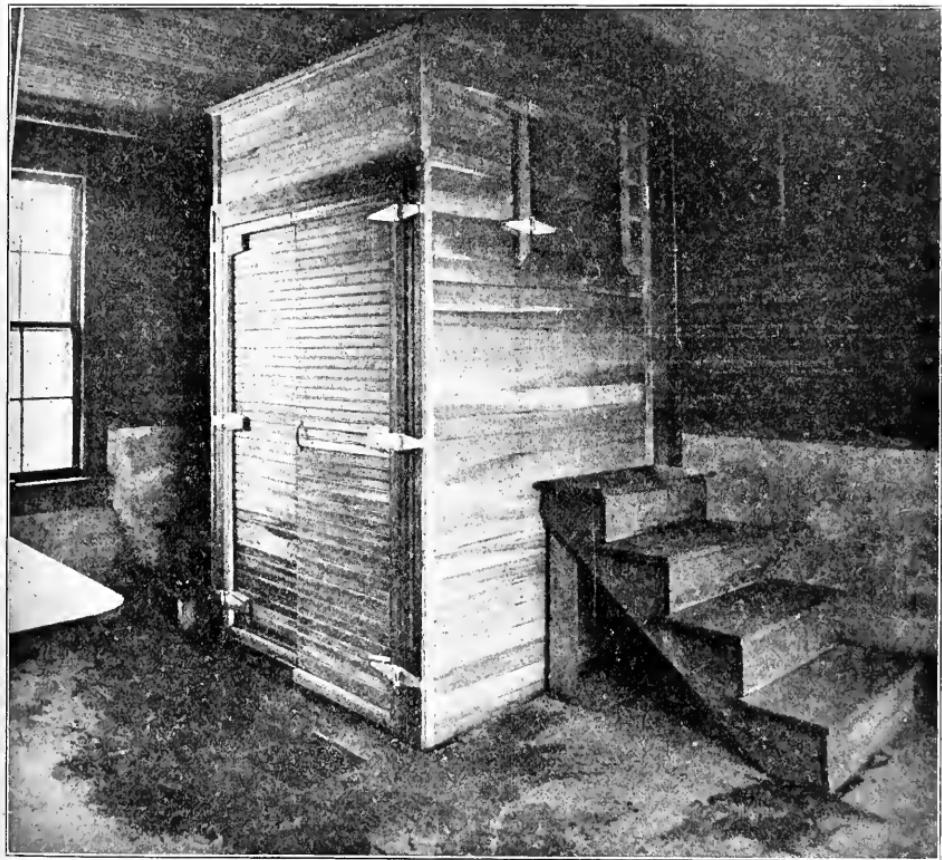


Fig. 2—Milk Cooler and Bottling Machine Boxed in.

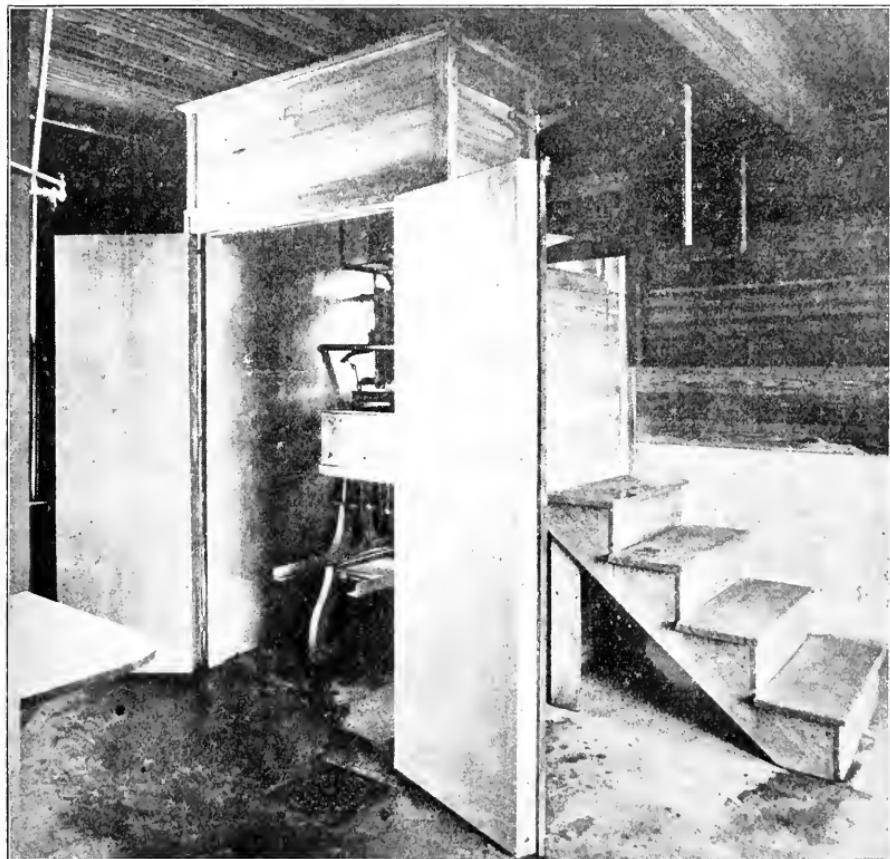


Fig. 3.—Doors Open Ready for Operation.

Fig. 4.—Milk Room, Walker Gordon Laboratory Co., Plainsboro, N. J.

Note the sanitary construction. Visitors are not allowed to enter this room. The milk cooler is exposed. A typical up-to-date plant.

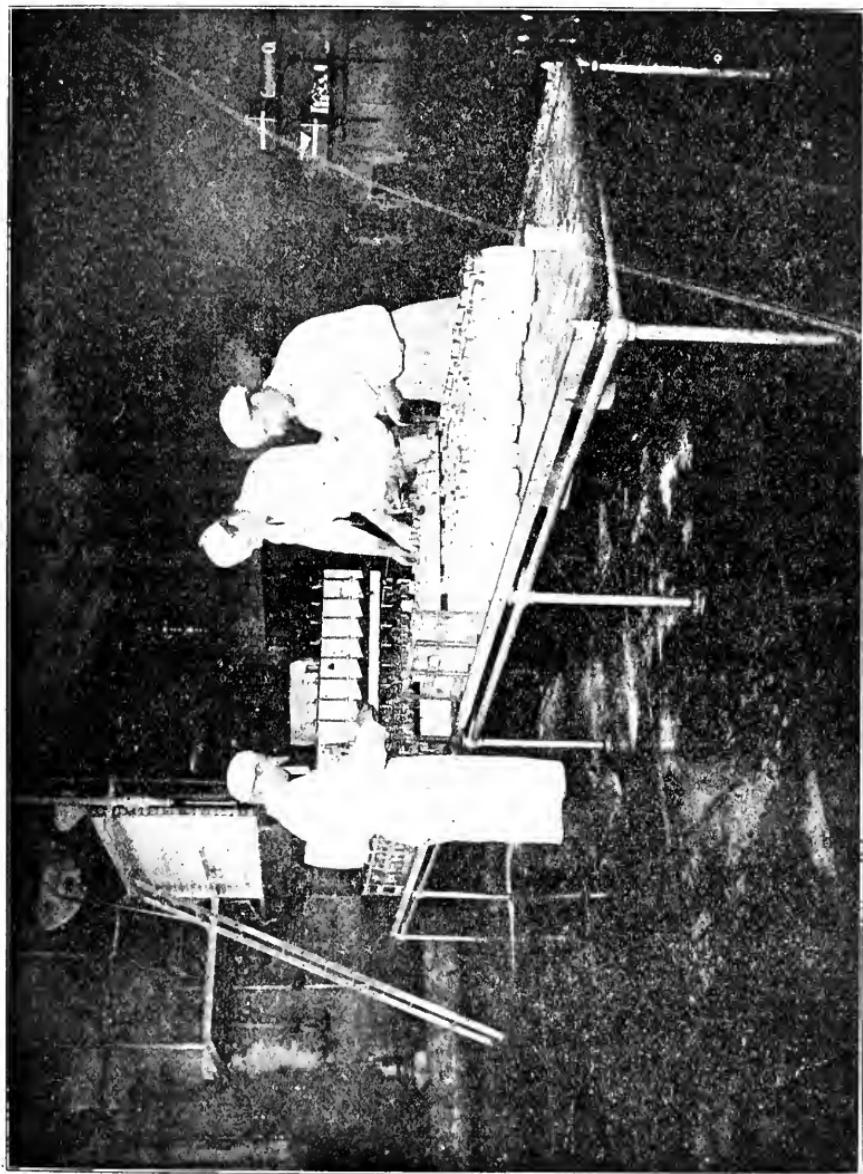


Table VII

	HIND QUARTERS		FRONT QUARTERS		95
	Left	Right	Left	Right	
Average weight of milk in grams.....	2,534	2,234	966	659	
*Approximate equivalent in cubic centimeters.....	2,448	2,158	933	643	
Average No. of bacteria per c. c.....	55	50	145	7,760	
Total bacteria in milk from quarter.....	134,600	105,900	135,300	4,990,000	

*The average specific gravity of milk is taken as 1.035 for this determination.

The above results indicate the extreme importance of watching not only the cow but each quarter of her udder in the production of high grade milk. For instance, if the milk from only the rear quarters is taken there is 10.5 pounds of very high grade milk carrying only about 53 bacteria per c. c., and if only that from the front quarters is taken there is 3.6 pounds of milk carrying about 3250 bacteria per c. c., while bringing together the milk from all four quarters we have 14.1 pounds of milk with a bacterial content of about 870 per c. c.

In conducting our experimental work with other cows similar variations were found in the milk from the different quarters, (see tables V, VIII, IX, X, and XIII). Some further investigations would be especially desirable along this line, but from the results of our work, we believe that it would pay the producers of certified milk to make frequent tests of the bacterial content of each quarter of every cow's udder and use the milk having a high germ content for purposes other than for direct consumption.

CAN THE HIGH GERM CONTENT OF A CERTAIN QUARTER OF A Cow's UDDER BE REDUCED?

In hope of finding some practical method to reduce the number of bacteria present in any given quarter of the udder, the three series of experiments described below were undertaken.

I. Sealing the opening of the teat so as to exclude possible external contamination.

After the udder had been thoroughly stripped the end of the teat to be sealed was dried with a sterile towel and then moistened with 25 per cent lysol. This was wiped off and carbolated vaseline rubbed onto the end of the teat. To protect the vaseline pieces of adhesive tape, three or four inches long were used so as to completely cover the end of the teat. The teats were sealed after every milking, but samples were secured only from the morning's milk and were taken in sterile glass stoppered bottles as previously described.

The following table gives the results of the tests:

Table VIII Teat Sealing Experiment

COW No. 4

DATE	SEALED QUARTER		OPEN QUARTER		Remarks
	Dilutions Used	Bacteria per cc.	Dilutions Used	Bacteria per cc.	
January 27, 1910.....	10 & 100	21200	10 & 100	400	Front quarter sealed.
January 28, 1910.....	10 & 100	2800	10 & 100	400	Front quarter sealed.
January 29, 1910.....	10 & 100	100	10 & 100	100	Front quarter sealed.
January 31, 1910.....	10 & 50	3300	10 & 50	250	Front quarter sealed.

COW No. 9					
	SEALED	QUARTER	OPEN	QUARTER	Remarks
	Dilutions Used	Bacteria per cc.	Dilutions Used	Bacteria per cc.	
February 1, 1910.....	10 & 50	900	10 & 50	10	Front quarter sealed.
February 2, 1910.....	10 & 50	700	10 & 50	65	Front quarter sealed.
February 3, 1910.....	10 & 50	1200	10 & 50	75	Front quarter sealed.
February 4, 1910.....	5 & 50	1500	5 & 50	40	Front quarter sealed.
February 5, 1910.....	5 & 25	320	5 & 25	30	Front quarter sealed.
February 7, 1910.....	5 & 25	320	5 & 25	110	Front quarter sealed.
February 9, 1910.....	5 & 25	440	5 & 25	200	Front quarter sealed.
February 11, 1910.....	5 & 25	440	5 & 25	60	Front quarter sealed.
February 14, 1910.....	5 & 25	110	10 & 25	1360	Rear quarter sealed.
February 16, 1910.....	5 & 25	130	5 & 25	1110	Rear quarter sealed.
February 17, 1910.....	5 & 25	20	5 & 25	2100	Rear quarter sealed.
February 18, 1910.....	5 & 25	40	5, 10 & 25	1170	Rear quarter sealed.
February 21, 1910.....	5 & 25	30	5 & 25	1910	Rear quarter sealed.

It would seem that carefully sealing the teat with carbonated vaseline after milking reduced the number of bacteria somewhat, but such a procedure could hardly be recommended without a more complete series of tests.

II Injecting the udder with dioxygen in order to destroy, wash out or hinder the development of bacteria.

For this work flasks of sterile distilled water were prepared as follows: Some 300 c. c. flasks were used, and 250 c. c. of distilled water poured into each, they were then set on a table and the height of the water was carefully marked on the outside, then 50 c. c. of water were poured out, the flasks plugged with cotton, and sterilized in an autoclave at 15 pounds steam pressure for 15 to 20 minutes. When about to be used, Dioxygen was added to the sterile water till the fluid was even with the marks on the side of the flask, thus making an approximate 20 per cent solution.

The injections were carried out as follows,—A milking tube was connected by a rubber tube to a wash bottle provided with a compression bulb so that the contents of the bottle could be forced through the milking tube. Before use the entire apparatus was sterilized by boiling. Before the injections were made the end of the teat was moistened with 25 per cent lysol. Then the milking tube was inserted into the milk duct and connections made with the wash bottle to which the solution had been transferred. In these tests the solution was used at ordinary room temperature. As the dioxygen seemed to be somewhat irritating it was milked out as completely as possible after it had remained in the udder for one minute. Injections were made on March 29th and 30th in the evening, and the samples of milk taken the next morning.

The following table gives the results of the tests:

Table IX Dioxygen Treatment Results

Date	HIND QUARTERS				FRONT QUARTERS				Remarks
	LEFT		RIGHT		LEFT		RIGHT		
	Dilutions Used	Bacteria per cc.							
Mar. 26, '10	5 & 25	26	5 & 25	32	10 & 50	90	50 & 250	3500	
Mar. 29, '10	5 & 25	26	5 & 25	32	5 & 25	50	50 & 125	9900	Right Front injecte with dioxygen Mar 29.
Mar. 30, '10	5 & 25	35	2, 9 & 50	490	Milk noticeable yellow (6 plates). Treated Mar. 30 as be fore. Milk very ye low.
Mar. 31, '10	5 & 25	40(a)	2 & 25	335(b)	
Apr. 1, '10	5 & 25	25	2 & 25	610	Milk quite yellow.
Apr. 2, '10	5 & 25	45	25 & 125	39000	Milk noticeably yellow.
Apr. 6, '10	9 & 25	160	50 & 250	4600	Milk normal color.
Apr. 7, '10	5 & 25	145	50 & 250	8000	Milk normal color.

(a) Milk curdled in 48 hours.

(b) Milk was not curdled after 7 days.

The above treatment produced a remarkable diminution in the number of bacteria, but the fact that it causes irritation renders it useless, since the numbers shortly after ceasing the injections are vastly greater than they were in the first place, and the quarters became slightly inflamed following the treatment.

III Injecting the udder with glycothymoline for the purpose of destroying, washing out or hindering development of bacteria.

Flasks of sterile distilled water were used in this work, and were prepared similar to those described in the Dioxygen experiments, 200 c. c. of water being placed in the flask to start with and the final solution of Glyco-Thymoline being 25 per cent. The solution was injected at blood heat immediately after milking in the evening. It was allowed to remain in the udder for one hour, and was then milked out as completely as possible.

Table X Glycothymoline Treatment and Results

DATE	HIND QUARTERS				FRONT QUARTERS				Remarks
	LEFT		RIGHT		LEFT		RIGHT		
	Dilutions Used	Bacteria per cc.	Dilutions Used	Bacteria per cc.	Dilutions Used	Bacteria per cc.	Dilutions Used	Bacteria per cc.	
June 7, '10	20 & 100	No colonies on plates	20 & 100	70	100 & 500	300	100 & 500	9150	
June 8, '10	10 & 100	10 & 100	10 & 100	20	20 & 100	45	20 & 100	8170	
June 9, '10	20	40	20	170	20 & 100	100	20 & 100	3320	R. F. Quarter Injected evening June 9.
June 10, '10	10 & 100	205	10 & 100	610	Injected as previously.
June 11, '10	10 & 100	145	5 & 50	1610	Injected as previously.
June 12, '10	5 & 50	380	5 & 50	1040	Injected as previously.
June 13, '10	5 & 50	145	5 & 50	1260	Injected as previously.
June 14, '10	5 & 50	85	5 & 50	3230	
June 15, '10	5 & 50	60	5 & 50	8930	Two R. F. plates had great No. of colonies.
June 16, '10	5 & 50	50	5 & 50	50	
June 18, '10	5 & 100	410	5 & 100	41800	100, 100000	130	10 & 100	5700	
Nov. 14, '10	5 & 100	410	5 & 100	31200	100 & 20000	183500	5, 100	64400	
Nov. 15, '10	5 & 100	3340	5 & 100	3340	100 & 20000	233600	100 & 20000	34600	
Nov. 16, '10	4 & 100	1710	10 & 200	16400	100 & 20000	623900	100 & 20000	45500	
Nov. 17, '10	5 & 50	1530	10 & 200	16400	100 & 20000	326600	100 & 20000	2227000	
Nov. 18, '10	5 & 100	1460	10 & 200	16400	100 & 20000	370000	500 & 5000	109700	{ L. F. and R. F. Injected evening Nov. 20.
Nov. 19, '10	5 & 50	4460	10 & 200	16400	100 & 20000	20300	20 & 20000	7500	"
Nov. 20, '10	5 & 50	6100	10 & 200	6100	100 & 20000	17500	50 & 500	5800	"
Nov. 21, '10	5 & 50	2300	10 & 200	2300	100 & 20000	39100	50 & 500	13500	"
Nov. 22, '10	5 & 50	6000	10 & 200	6000	100 & 20000	1974000	50 & 500	8400	"
Nov. 23, '10	5 & 50	1700	10 & 200	1700	100 & 20000	50 & 500	50 & 500	34700	No injection last evening.
Nov. 24, '10	5 & 50	50	10 & 200	50	50 & 500	50 & 500	50 & 500	50 & 500	19000
Nov. 25, '10	5 & 50	50	10 & 200	50	50 & 500	50 & 500	50 & 500	50 & 500	4040000
Nov. 26, '10	5 & 50	1000	10 & 200	1000	100 & 500	24000	50 & 500	3790000	100 & 500
Nov. 27, '10	100	2400	10 & 200	2400	100 & 500	3560000	100 & 5000	1855000	100 & 10000
Dec. 1, '10	10 & 100	2400	10 & 100	2400	100 & 10000	100 & 10000	100 & 10000	100 & 10000	

Here again it is evident that there was a considerable reduction in the number of bacteria from injected quarters, but that after the treatment had been stopped the numbers soon became as great or greater than they were before. During the first series of trials, June, 1910, there was no sign of injury to the udder as a result of the injections, and it had been planned to have the second series extend over a much longer period of time, but after four injections the quarter used was showing some inflammation and the treatment had to be stopped. As a result of the irritation induced at this time, the bacterial content of that quarter rose to 4 million per c. c. within three days after the last injection was used. The inflammation gradually subsided in three or four days after the injections were discontinued.

While these experiments did not give us the desired results, we believe that a great deal more work should be undertaken along these lines, and that something of considerable practical value will eventually be learned.

HOW NEARLY GERM-FREE CAN MILK BE OBTAINED?

If the bacterial content of the milk from each quarter be determined, and those quarters selected which usually run low in germs, how nearly germ-free can milk be produced commercially? A few tests were carried out with the cow used in the teat sealing experiment, the milk from the rear quarters only being saved. The first few streams were rejected from these quarters and the remainder milked into a sterile pail provided with a small top and held in a somewhat horizontal position while milking. The very last part of the stripplings was not included in the samples. The following table gives the details of these tests. Each sample consisted of about two quarts of milk.

Table XI Results of Experiment to Determine How Nearly Sterile Milk Can be Drawn from Udder

DATE	Dilutions Used	Bacteria per cc.	Remarks
February 5, 1910...	1 & 10	120	6 plates poured.
February 7, 1910..	1 & 2	6	
February 11, 1910...	1 & 2	8	
February 14, 1910...	1 & 2	19	
February 16, 1910...	1 & 2	25	
February 26, 1910..	1 & 2	35	Only 2 plates poured.

Average number of bacteria per cc. 35.

These six samples of milk contained an average of 35 bacteria per c. c. That there remains vast opportunity for improvement in the production of more sanitary milk on the part of dairymen is shown by comparing these results with the figures given in the following table which gives the average number of bacteria per c. c. found in the commercial milk supplied to the city of Rochester, N. Y., during the years 1900 to 1904, inclusive. In this connection it should be remembered that Rochester takes high rank among the cities of this country in respect to the purity of its milk supply, due to the efficient supervision exercised by its officials.

Table XII Average Number of Bacteria Per cc. Found in Milk at Rochester, N. Y. *

Month	1900			1901			1902			1903			1904				
	Total Cultures per cc.	Average per cc.	Over 5,000,000 Under 100,000														
January.....	755,971	25	5	296,100	24	9	191,989	48	1	17	190,272	46	26	164,081	44	21	
February.....	137,361	29	12	293,839	25	2	155,948	44	2	24	96,105	40	1	106,462	48	32	
March.....	189,424			176,200	24	10	41,975	47	3	27	220,812	42	2	256,363	46	1	
April.....				402,735	24	7	345,609	45	2	163,166	45	2	23	226,958	43	1	
May.....				415,504	24	8	240,9,651	48	3	14	189,825	47	8	303,186	45	2	
June.....				468,066	23	4	258,136	47	5	18	280,628	43	3	315,827	44	3	
July.....				326,285	24	4	5	232,614	46	2	5	174,151	29	3	328,231	45	3
August.....				322,285	24	4	3	243,558	47	4	9	362,357	28	2	577,204	45	1
September....				325,325	24	5	11	298,996	23	2	1	209,655	29	4	259,845	31	15
October.....				161,029	24	6	139,461	23	13	147,955	46	2	21	359,372	47	1	
November....				103,959	24	2	10	100,814	42	2	24	110,223	47	1	213,287	48	33
December....				195,422	24	1	9	163,789	48	18	95,047	49	41	181,397	47	30	
														111,888	48	27	
Average....	1,403,191	24	33	10%	15%	55	275,327	287	9%	28%	215,917	531	26	34%	38%	47%	
	796,468	319	33				28	82			185	492	15	187	253,727	11	253

BACTERIA IN THE FIRST MILK TAKEN FROM A COW

It seemed desirable to learn whether or not bacteria were present in colostrum, or first milk, drawn from a heifer with her first calf. The calf was removed before it had opportunity to suck and the samples secured in the usual way.

The dilutions used for the first set of samples were 1 to 1, 1 to 100 and 1 to 1000; for the second set, 1 to 1 and 1 to 100; and for the third and fourth sets 1 to 2 and 1 to 10. The results are given below:

Table XIII

Date	HIND QUARTERS		FRONT QUARTERS	
	Left	Right	Left	Right
	Bacteria per cc.	Bacteria per cc.	Bacteria per cc.	Bacteria per cc.
Feb. 21, 1910.....	150	190	220	95
Feb. 22, 1910.....	180	75	100	35
Feb. 24, 1910.....	40	60	220	70
Feb. 26, 1910.....	210	460	3350	1400

We had presumed that there would either be a very large number or else practically none, as they would be expected to multiply very rapidly after they had gained entrance, and were not being removed by milking. In this case, however, there was just an average number of organisms present, and they showed a greater tendency to increase in the front quarters than in the hind. In the case of every cow with which we have made tests involving front and rear portions of the udder separately, a very much higher count has been found in milk from the front than in milk from the hind quarters. We know that some other investigators have not secured similar results and doubtless a very large series of tests would be required in order to obtain reliable data on that point.

The experimental results given in this bulletin are considered as largely in the nature of a preliminary report. It is believed, however, that some valuable practical information has been secured, and that some of the data given, may be of service in future investigations.

